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Additions to *Ramularia* species (hyphomycetes) in Poland

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Abstract — The morphology and revised distribution of three *Ramularia* species (teleomorphs unknown) are presented based on fresh specimens. *Ramularia melampyri* is new for Poland, and *R. celastri* is reported from its third (and easternmost) locality in Europe. *Ramularia abscondita* specimens confirm the occurrence of this species in Poland. As *R. melampyri* hosts (*Melampyrum* spp.) are currently classified in *Orobanchaceae,* the implications of the new systematics of *Scrophulariaceae* s.l. for the taxonomy of *Ramularia* and related *Mycosphaerella* species are discussed briefly.

Key words — microfungi, asexual morphs, plant parasites, biogeography, new records

Introduction

The genus *Ramularia*, described by Unger in 1833 (cf. Braun 1998), is one of the largest anamorph genera, with known teleomorphs classified in the ascomycetous genus *Mycosphaerella* Johanson (Braun 1998, Crous 2009). *Mycosphaerella* s.l. is polyphyletic, but its type species, *M. punctiformis* (Pers.) Starbäck, has a proven *Ramularia* anamorph (*R. endophylla* Verkley & U. Braun). Consequently, the name *Mycosphaerella* s.str. has been confined to sexual morphs associated with *Ramularia* anamorphs (Verkley et al. 2004, Crous et al. 2009a). According to the new rules of Art. 59 of the ICN (McNeill et al. 2012), *Mycosphaerella* and *Ramularia* are heterotypic synonyms, and so the older name *Ramularia,* which has priority, is now a holomorph name.

Ramularia species have been assumed to form a single lineage within the *Mycosphaerellaceae*. The recently described *Mycosphaerella cerastiicola* Crous, which clusters in the middle of the *Ramularia* clade, has a septoria- or pseudocercosporella-like anamorph (Crous et al. 2011), which casts reasonable doubt on the monophyly of *Ramularia*. Unfortunately, *M. cerastiicola* has not been included in the recent studies of *Septoria* and septoria-like genera (Quaedvlieg et al. 2013, Verkley et al. 2013).

The genus has been the subject of thorough taxonomic studies worldwide (Braun 1998, Crous et al. 2001, 2009a, 2011; Braun & Pennycook 2003, Braun

et al. 2005, Zhang 2006, Kirschner 2009). The Polish monograph covered 115 *Ramularia* species (Wołczańska 2005). The number of taxa known in Poland was further extended with *Ramularia celastri* (Czerniawska 2007), *R. epilobiana* (Sacc. & Fautrey) B. Sutton & Piroz. (Ruszkiewicz-Michalska 2006), and *R. vallisumbrosae* Cavara (Wołczańska 2010). In addition, ecological data on two very rare species (*R. agrimoniae* Sacc. and *R. uredinearum* Hulea) have been reported in Poland (Ruszkiewicz-Michalska et al. 2012), and a new host plant (*Hypochoeris radicata* L.) has been listed for *R. inaequalis* (Preuss) U. Braun (Wołczańska & Wołkowycki 2010).

Materials & methods

Plant organs affected by *Ramularia* species were collected in 2007 and in 2012. *Ramularia melampyri* and *R. abscondita* were found during systematic studies of plant-parasitic fungi in the Las Łagiewnicki forest complex in the city of Łódź (central Poland). This forest complex is under strong anthropogenic pressure, as many residents use it for recreational purposes (Kurowski 2001). We found a single specimen of *R. celastri* in Biebrza National Park (NE Poland); this largest and among the newest Polish national parks is preserving the whole river valley with its natural longitudinal and transversal zonation of habitats and corresponding plant communities (Dyrcz & Werpachowski 2005) as well as numerous interesting and endangered fungi (Kujawa et al. 2012). Geographical coordinates were obtained with Garmin's GPSmap 60 device. Collector abbreviations include MRM = Ruszkiewicz-Michalska, EP = Połeć, and AK = Agnieszka Kuchnik.

Fresh specimens were mounted in lactophenol picric acid solution (Fluka) and examined using a Nikon Eclipse 50i microscope; measurements of morphological structures were made also in tap water. Microphotographs were taken with a Nikon DS-F1 digital camera using phase contrast.

The fungi were identified based on Braun's (1998) monograph, and their host plants were determined using the key by Rutkowski (2004). The fungal nomenclature agrees with Index Fungorum (2014) while the host nomenclature follows Mirek et al. (2002) and The International Plant Names Index (2014). The study material is deposited in the subcollection Parasitic Fungi (labeled as PF) of the Fungal collection of Herbarium Universitatis Lodziensis (LOD).

Our attempts to isolate and cultivate the three reported species failed despite using standard methods (Crous et al. 2009b, Verkley et al. 2004). Also, the enrichment of culture media (WA, MEA, CMA, OA, Difco) with autoclaved host leaves elicited no positive effect.

Species characteristics

Ramularia melampyri Ellis & Dearn., Canad. Record Sci. 5: 271 (1893) Pl. 1

Leaf spots very inconspicuous, diffuse discolorations, pale greenish, later brownish green. Conidiophores in small to fairly large, loose to dense, cream-colored fascicles, scattered at the leaf spot area, arising from stromata, straight,

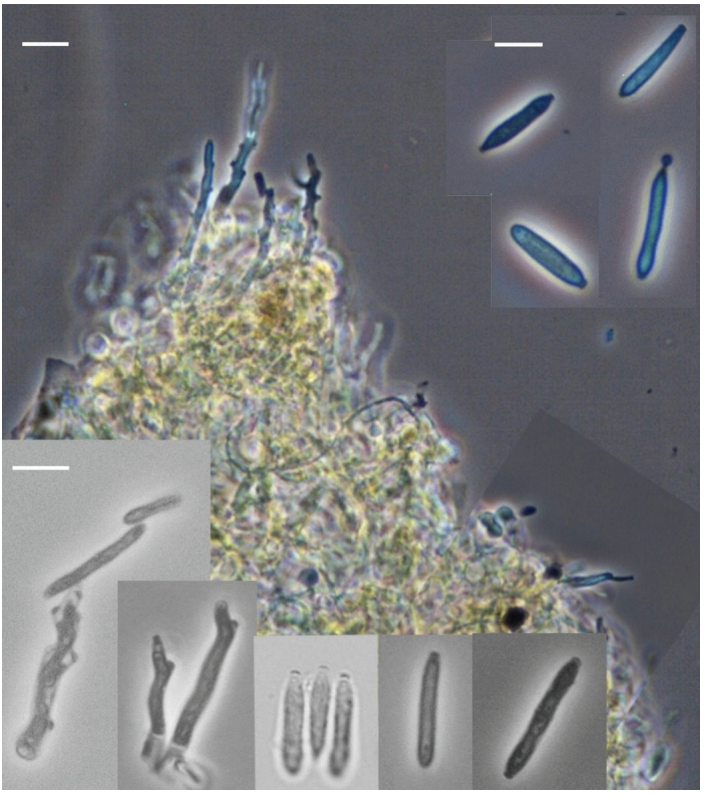

Plate 1. *Ramularia melampyri* (LOD 3383): conidiophores and conidia (lactophenol picric acid solution; phase contrast). Scale bars = 10 µm.

subcylindric to flexuous, filiform, geniculate-sinuous, simple, 20–42.5 × 2–3.7 µm [5–100 × 2–6.5 µm; Braun 1998], continuous to septate, hyaline, smooth; conidial scars slightly thickened and darkened. Conidia catenate, ellipsoid-ovoid, subcylindric-fusiform, (8.5–)10–24.5 × 2.5–5 µm [(9–)12–28(–30) × (3–)4–6.5(–8) µm; Braun 1998], 0–1-septate, hyaline, smooth, ends obtuse to somewhat attenuated; hila somewhat thickened, darkened.

Specimens examined — On *Melampyrum nemorosum* L.: **POLAND**, **Łódź Voivodeship**, Łódź, Las Łagiewnicki forest, roadside, 51°49′59″N 19°28′10″E, 28 July 2007, leg. MRM (LOD 3383); 08 Aug. 2012, leg. EP (LOD 3384).

Ecology & distribution — This species has been recorded on eight *Melampyrum* species [*Orobanchaceae*] from North America and Eurasia (Braun 1998, Farr & Rossman 2014, Negrean & Denchev 2000, Pando et al. 2003, GBIF 2014a).

Ramularia melampyri has no known teleomorphic or synanamorphic connections (Tomilin 1979, Braun 1998, Aptroot 2006, Anon. 2014).

Comments — *Scrophulariaceae* s.l., which has undergone revision based mainly on molecular studies, has been split into four families (cf. Stevens 2014). Consequently, host-based keys to *Ramularia* species on *Scrophulariaceae* s.l., such as those by Braun (1998) and Wołczańska (2005), may need some reconstruction. A summary of the host-pathogen relationships of these *Ramularia* species, disposed under the four segregate host families, is presented in Fig. 1.

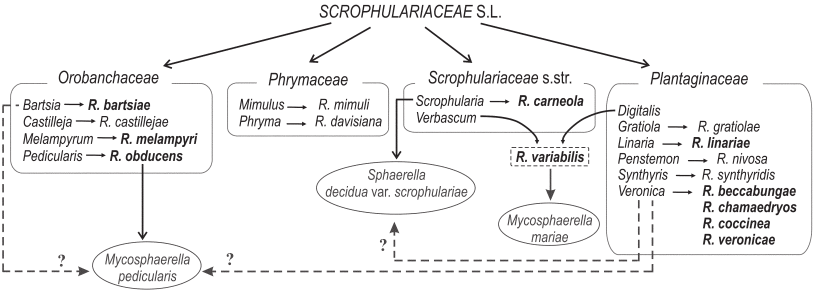

Figure 1. Host-parasite relationships in *Scrophulariaceae* s.l. (*Ramularia* species occurring in Poland set in bold font; teleomorphs are given within ellipses). Survey based on plant phylogeny updates (Stevens 2014) and on mycological data given by Braun (1998), Braun & Scheuer (2008), Tomilin (1979), Aptroot (2006), and Anon. (2014).

The difficulty with host-based taxonomy of *Ramularia* is most evident with *R. variabilis* Fuckel, originally described by Fuckel (1870) from both *Verbascum* [*Scrophulariaceae* s.str.] and *Digitalis* [*Plantaginaceae*], and subsequently lectotypified on *Verbascum* (Braun 1998). The *Mycosphaerella* names tentatively associated with *Ramularia* species on *Scrophulariaceae* s.l. also indicate a high degree of uncertainty (Fig. 1), involving hosts from more than one of the new segregate host families. The molecular relationships between *Ramularia*/*Mycosphaerella* species associated with *Scrophulariaceae* s.l. are largely unexplored. *Ramularia coccinea* (Fuckel) Vestergr. is the only taxon for which rDNA sequences have been deposited in GenBank (accessed 15 March 2014).

Ramularia abscondita (Fautrey & Lambotte) U. Braun, Int. J. Mycol. Lichenol. 3: 280 (1988) Pl. 2

Leaf spots angular-irregular, 1–10 mm diam., yellowish-ochraceous to light to medium brown, confluent, margin brown, narrow or indefinite.

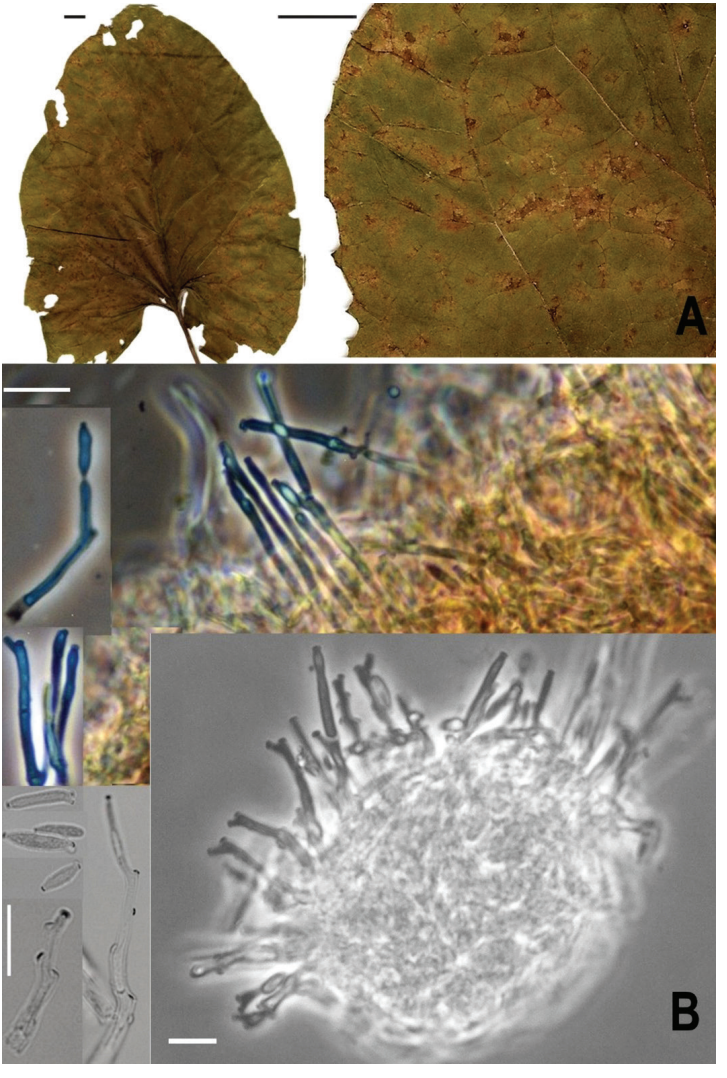

Plate 2. *Ramularia abscondita* (LOD 3218): A – a leaf of host plant with symptoms of fungal infection; B – conidiophores and conidia (lactophenol picric acid solution; phase contrast). Scale bars: A = 1 cm; B = 10 µm.

Conidiophores in small, loose fascicles, on the upper leaf surface, straight, subcylindric to geniculate-sinuous, mostly simple, (9.5–)11.25–25(–27) × 1.25–2.5 µm, septate, hyaline and smooth; conidial scars somewhat thickened and darkened. Conidia catenate, rarely in short, branched chains, ellipsoid,

subcylindric, (6.5–)7.5–17.5 × 2.5(–3) µm, aseptate, occasionally 1-septate, hyaline, smooth, ends obtuse to subacute; hila somewhat thickened, darkened.

Specimens examined — On *Arctium lappa* L.: **POLAND**, **Łódź Voivodeship**, Łódź, Las Łagiewnicki forest, roadside, 51°50′06″N 19°28′06″E, 28 July 2007, leg. MRM (LOD 3362); Gać Spalska nature reserve, forest road close to the Gać river, 51°34′31″N 20°08′01″E, 11 Aug. 2012, leg. MRM (LOD 3363).

On *Arctium nemorosum* Lej.: **POLAND**, **Łódź Voivodeship**, Łódź, Las Łagiewnicki forest, oak-hornbeam forest (*Tilio-Carpinetum calamagrostietosum*), roadside, 51°50′02″N 19°28′21″E, 28 July 2007, leg. AK (LOD 3218); 25 Aug. 2007 (LOD 3217).

On *Arctium tomentosum* Mill.: **POLAND**, **Łódź Voivodeship**, Łódź, Las Łagiewnicki forest, opening near Building of Forestry Unit, 51°50′26″N 19°28′18″E, 28 May 2012, leg. MRM (LOD 3450).

Ecology & distribution – This species has been reported on six *Arctium* species (*Asteraceae*) in Asia and Europe, including Poland (Braun 1998, Farr & Rossman 2014, GBIF 2014b). However, the Polish reports given by Braun (1998) were probably based on old records or collections and remain unclear and unproven (Braun, pers. comm.). *Ramularia abscondita* was not included in the Polish *Ramularia* monograph (Wołczańska 2005) or checklist of Polish micromycetes (Mułenko et al. 2008). We also have found no specimens or literature to confirm the previous Polish records.

Ramularia abscondita has no known teleomorphic or synanamorphic connections (Tomilin 1979, Braun 1998, Aptroot 2006, Anon. 2014).

Ramularia celastri Ellis & G. Martin, Amer. Naturalist 16: 1003 (1882) Pl. 3
= *Ramularia monachorum* Bubák, Bot. Közl. 14: 79 (1915)

Leaf spots subcircular to irregular, dark greenish, later greyish-ochraceous, margin narrow, reddish brown. Conidiophores hypophyllous, in small, loose to dense fascicles, arising from stromata, geniculate-sinuous, simple, (8.5–)10–45 × 1.5–3.5(–4) µm, continuous to 1-septate, hyaline, smooth; conidial scars slightly thickened and darkened. Conidia mostly catenate, subcylindric to fusiform, 9.5–30(–32) × 2.5–4.5 µm, 0–2-septate, hyaline, smooth, subobtuse; hila somewhat thickened, darkened.

Specimen examined — On *Euonymus europaea* L.: **POLAND**, **Podlaskie Voivodeship**, Biebrza National Park, Kapice protective unit, oak-hornbeam forest (*Tilio-Carpinetum*), 53°32′14″N 22°43′13″E, 28 Aug. 2012, leg. MRM (LOD 3451) [together with *Asteromella euonymella* (Sacc.) Aa & Vanev, *Septogloeum carthusianum* (Sacc.) Sacc.].

Ecology & distribution — This species has been reported mostly from North America and Asia (mainly China) on diverse *Celastrus* and *Euonymus* species (Braun 1998, Farr & Rossman 2014, GBIF 2014c, Cybertruffle's Robigalia 2014). In Europe it is known only from three collections on *E. europaea*: Bubák's holotype of *R. monachorum* from the Piva Monastery in northern Montenegro

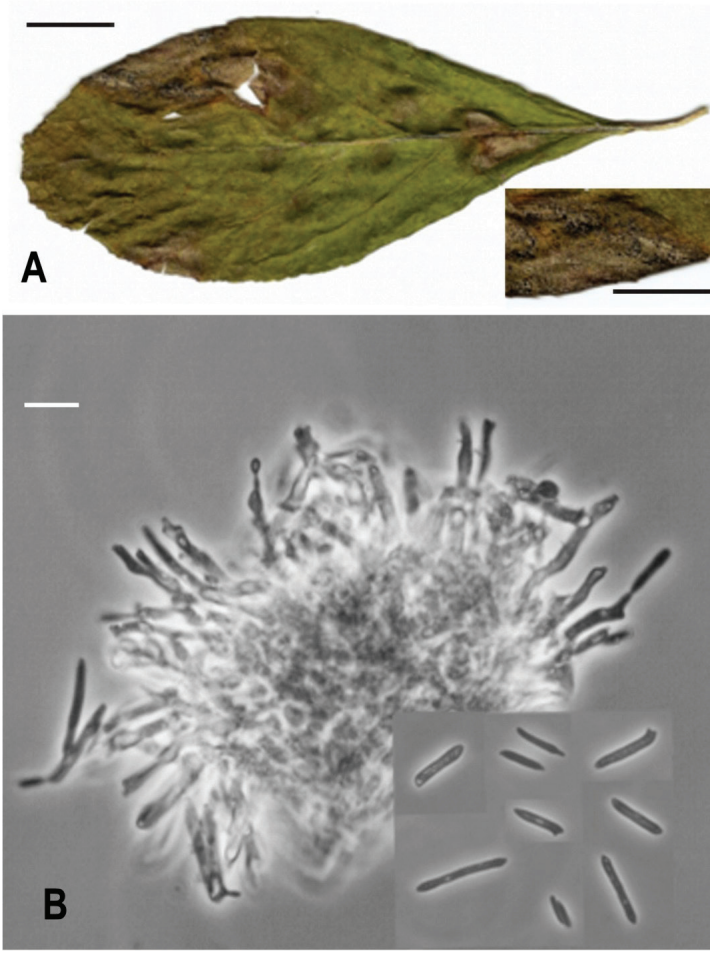

Plate 3. *Ramularia celastri* (LOD 3451): A – a leaf of host plant with symptoms of fungal infection; B – conidiophores and conidia (lactophenol picric acid solution; phase contrast). Scale bars: A = 1 cm; B = 10 µm.

(Farr & Rossman 2014); a report from Drawa National Park in northwestern Poland (Czerniawska 2007); and our new record, which extends the European range of this species to eastern Poland.

Ramularia celastri has no proven teleomorphic or synanamorphic connections (Tomilin 1979, Braun 1998, Aa & Vanev 2002, Aptroot 2006, Anon. 2014).

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